GENE THERAPY

Directed evolution of designer vectors

Gene therapy has been hampered by problems that have limited its clinical progress, including inefficient gene delivery and safety concerns. Designing better delivery vectors is therefore a paramount requirement for this technology to realize its potential. In a paper published in *Nature Biotechnology*, David Schaffer and colleagues describe a high-throughput method to aid the design of viral vectors with improved functional properties.

Recombinant adeno-associated viral vectors (rAAV), which are non-pathogenic and can establish persistent transgene expression in various cell types, are at the forefront of vector design efforts yet present a number of therapeutic challenges. Problems associated with rAAV delivery stem from the properties of the outer shell of the virus, the capsid, which is a major focus of engineering strategies. In past studies function-enhancing peptide sequences were inserted into specific sites in the capsid gene, providing improved cell-specific targeting. However, modifying more complex vector activities by this approach would require a greater understanding of the relationship between capsid structure and specific properties. This new study bypasses this issue by exploiting an approach that has been successful in protein engineering: directed evolution.

The authors generated more than 10^6 AAV2 variants with random mutations throughout the capsid gene. The resulting library was screened for variants with desirable functional properties followed by further rounds of mutagenesis and screening to enrich the selection. Proof of principle was demonstrated by applying the technique to two clinically relevant problems. One issue is that binding to heparin sulphate can limit rAAV2 dispersal. Affinity chromatography was used to identify variants with low heparin-binding affinity, additionally confirming the library's high functional diversity. Another major therapeutic limitation is that patients previously exposed to wild-type AAV2 carry antibodies that bind to and inactivate the vector before it can reach its target. Several variants were identified that could avoid antibody neutralization *in vitro*. These mutants were used to produce a vector with better delivery efficacy and less antibody neutralization than wild-type vector *in vivo*.

IMMUNOMODULATORS

Reversing exhaustion

Feeling the effects of a relentless and overloaded schedule? T cells also suffer from exhaustion and are less effective at doing their job when faced with persistent antigen, such as during chronic viral infection or cancer. Now, researchers reporting in *Nature* describe a mechanism that reverses the $CD8^+$ T-cell exhaustion seen in chronic infection of mice with lymphocytic choriomeningitis virus (LCMV) and restores the ability of T cells to control the virus.

LCMV is a natural pathogen of mice and, importantly for this study, strains are available that cause either acute (the Armstrong strain) or chronic (the clone 13 strain) infection. Therefore, infection with the Armstrong strain is cleared within a week and a stable pool of long-lived memory T cells is established. By contrast, the clone 13 strain establishes a persistent infection, which overwhelms the immune response and results in the generation of functionally impaired or exhausted virus-specific $CD8^+$ T cells.

> PDL1 blockade could reverse the functional impairment of the exhausted T cells and reduce the viral load.

To study T-cell dysfunction in chronic infection, Rafi Ahmed and colleagues first carried out gene-expression analysis of T cells that were generated following infection with each LCMV strain. Most notably, the exhausted virus-specific $CD8^+$ T cells contained more mRNA encoding the inhibitory receptor PD1 (programmed death 1; also known as PDCD1) than functional virus-specific $CD8^+$ T cells from Armstrong-strain-infected mice. Further analysis showed that although PD1 was transiently expressed by $CD8^+$ T cells after infection with the Armstrong strain, it was rapidly downregulated, whereas $CD8^+$ T cells responding to the clone 13 strain retained high PD1 expression throughout the chronic infection. Moreover, one of the ligands for PD1, PDL1, was shown to be highly expressed by persistently infected splenocytes, indicating that this inhibitory receptor–ligand interaction might regulate T-cell function during chronic LCMV infection.

To test this, the authors treated persistently infected mice with a blocking antibody specific for PDL1. Remarkably, compared with untreated mice, chronically infected mice that were treated with PDL1-specific antibody underwent a marked expansion of virus-specific $CD8^+$ T-cell populations, which had an increased ability to produce interferon-γ and tumour-necrosis factor, thereby reversing the exhausted phenotype. In addition, PDL1 blockade markedly reduced the viral load and resolved infection with the clone 13 strain.

The authors then showed that, even in mice that lack $CD4^+$ T cells and therefore suffer from a more severe form of chronic infection and T-cell exhaustion, PDL1 blockade could reverse the functional impairment of the exhausted T cells and reduce the viral load. This might be of particular relevance for HIV infection, which is characterized by a loss of $CD4^+$ T cells.

Although, on the basis of these results, blockade of the PD1–PDL1 pathway for the treatment of chronic viral infection or cancer is tantalizing, prolonged disruption of this regulatory pathway can result in autoimmunity, as exemplified by PDL1-deficient mice.

Lucy Bird, Nature Reviews Immunology

ORIGINAL RESEARCH PAPER Barber, D. L. *et al.* Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature* **439**, 682–687 (2006)
FURTHER READING Williams, M. A. & Bevan, M. J. Exhausted T cells perk up. *Nature* **439**, 669–670 (2006)

Although this approach cannot address issues such as the possible mutagenic effects of vector integration into a functional gene, it could apply to any properties governed by capsid structure and could help to overcome a variety of problems associated with gene delivery. A directed-evolution approach might lead to improvements in the specificity and efficiency of targeting particular cell types, which are influenced by interactions with cell surface receptors. Additionally, this strategy could help to reduce vector antigenicity. Furthermore, this approach can add to our understanding of the molecular basis of vector properties.

Katherine Whalley

ORIGINAL RESEARCH PAPER Maheshri, N. *et al.* Directed evolution of adeno-associated virus yields enhanced gene delivery vectors. *Nature Biotechnol.* doi:10.1038/nbt1182 (2006)
FURTHER READING Neylon, C. Chemical and biochemical strategies for the randomization of protein encoding DNA sequences: library construction methods for directed evolution. *Nucleic Acids Res.* **32**, 1448–1459 (2004).

PROTEIN–PROTEIN INTERACTIONS

Muscling in on p53

Modulating the activity of proteins by perturbing their interactions with coactivator and corepressor proteins is an attractive therapeutic approach, but effectively targeting the dynamic and complex interactions between proteins can be challenging (see Further Reading). Now, researchers in Ming-Ming Zhou's lab at Mount Sinai School of Medicine, New York, have developed a series of small-molecule inhibitors that prevent the association of p53 with its coactivator, the histone acetyltransferase p300/Creb-binding protein (CBP), and were able to downregulate the p53-mediated response to DNA damage.

DNA damage causes a sequence of regulatory events that culminates in lysine acetylation of p53, which enables it to bind and activate its target genes. Recent reports have suggested that rather than affecting the DNA-binding activity of p53 directly, lysine acetylation promotes the recruitment of p53 coactivators. Little is known, however, about the effects of individual lysine residues on p53 activity because lysine acetylation (AcK) sites are clustered together, making it difficult to study the effects of modification at specific sites. On the basis of previous knowledge that the p53 residue K382 is a binding site for the bromodomain (BRD) of p300/CBP, Zhou and colleagues set out to identify small-molecule binders of this domain that could disrupt the interaction between p300/CBP and p53 as a means to study the functional consequences of lysine acetylation at this site.

The authors first built a focused library of 200 drug-like compounds based on structural knowledge of BRD, and used NMR to screen for BRD-specific ligands. They identified 14 compounds, of which 13 showed selective binding for the BRD of p300/CBP compared with BRDs from other transcriptional proteins. Structural analysis revealed that the aromatic ring of almost all of the compounds bound to the hydrophobic AcK-binding site, and that most of the residues predicted to interact with the ligand were within the specific loops (ZA and BC) that form the hydrophobic pocket of the AcK-binding site.

The three-dimensional structure of the p300/CBP BRD bound to one of the compounds, MS7972, also showed a network of interactions between the ligand and CBP residues within the AcK-binding site, suggesting that MS7972 could block the interaction of the CBP BRD with its protein-binding partners.

The authors studied the compounds in a competition assay in which the BRD ligand competes with a biotinylated p53–AcK382 peptide for streptavidin-immobilized, glutathione-labelled CBP BRD. Incubation with 50–100 μM of the most potent compounds, MS7972 and MS2126, completely blocked the interaction between CBP BRD and p53–AcK382.

These two compounds were next evaluated in an assay of p53 transcriptional activation in human bone osteosarcoma epithelial cells. In this assay doxorubicin is added to cause DNA damage; the upregulation of p53 protein and associated p53-target genes such as p21 is then measured under different conditions. Treatment of resting cells with 200 μM of MS7926 or MS2126 before addition of doxorubicin dramatically decreased the doxorubicin-induced increase in cellular p53 and caused a decrease in p53-mediated p21 activation. Moreover, at different time points after addition of doxorubicin there was a loss of acetylation at K382.

Although further investigation is needed to confirm that these two compounds modulate p53 by blocking the interaction between CBP BRD and p53–AcK382, this study demonstrates a novel method for identifying small molecules that target protein–protein interactions that could be useful for analogous pathways involved in disease.

Joanna Owens

ORIGINAL RESEARCH PAPER Sachchidanand *et al.* Target structure-based discovery of small molecules that block human p53 and CREB binding protein association. *Chem. Biol.* **13**, 81–90 (2006)
FURTHER READING Arkin, M. R. & Wells, J. A. Small-molecule inhibitors of protein–protein interactions: progressing towards the dream. *Nature Rev. Drug Discov.* **3**, 301–317 (2004)
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